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# Catalepsy Induced by Striatal Acetylcholinesterase Inhibition With Fasciculin in Rats

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CASTELLÓ, M. E., B. BOLIOLI AND F. DAJAS. *Catalepsy induced by striatal acetylcholinesterase inhibition with fasciculin in rats.* PHARMACOL BIOCHEM BEHAV 41(3) 547–550, 1992.—The acetylcholinesterase inhibitor peptide fasciculin (FAS) was bilaterally injected into the striatum of rats. Twenty-four hours after injection, animals showed a cataleptic syndrome that was potentiated by haloperidol (HAL). The catalepsy was significantly decreased by IP atropine. Biochemically, only an increase of the homovanillic acid in the striatum was found 24 h and 7 days after FAS treatment. Seven days after the intrastriatal FAS injection, there was no HAL potentiation of catalepsy, which was even lower than that of rats treated with IP HAL after intrastriatal injection of saline. Results are interpreted as showing the central role of the cholinergic system in the induction of catalepsy in the rat.

Catalepsy Basal ganglia Fasciculin Cholinergic Toxin Haloperidol HVA

AN akinetic state with some similarities to those seen in patients with extrapyramidal disorders, catatonic schizophrenia, or after neuroleptic treatment of psychosis can be obtained in rodents after intraperitoneal haloperidol (HAL) administration (11,20). This syndrome, called catalepsy, has been used as an animal model for neuroleptic-induced Parkinsonism and also as an indicator of neuroleptic antipsychotic activity. Since HAL is a dopaminergic antagonist, the dopaminergic pathways of the basal ganglia were first related to this syndrome (5,6). Other neurotransmitter systems such as the GABAergic and glutamatergic ones have been also implicated (21,22). Furthermore, in the basal ganglia there is a well-known balance between two of their main neurotransmitter systems: the dopaminergic and cholinergic ones (2). Since arecoline and pilocarpine have a synergistic effect with HAL and the effects of arecoline and HAL combined or alone are blocked by atropine sulfate, the cholinergic system has been also implicated in the induction of catalepsy (7). Nevertheless, the role of the cholinergic system is not as well established as the dopaminergic one since, for example, doses of pilocarpine required to induce catalepsy are very large (13). Besides, the role of the enzymes of the cholinergic pathway like acetylcholinesterase (AChE) in the induction of catalepsy is not totally known in spite of the fact that an anticholinesterase drug such as physostigmine appears to produce catalepsy and increase neuroleptic-induced Parkinsonism (17,20).

An important and recent body of data (9,19) has provided evidence that AChE is playing a noncholinergic role in the substantia nigra (SN). Since SN AChE is found in the cell bodies of the dopaminergic neurones of the nigrostriatal pathway, it is conceivable that functions other than the cholinergic one could also be responsible for the anticholinesterase-induced catalepsy in the rat.

In this context, we performed bilateral intrastriatal injections of a new anticholinesterase peptide, called fasciculin (FAS), that inhibits both the cholinolytic and peptidase activities of AChE (4,8). We tested FAS cataleptic activity and its potentiation by HAL, as well as its blockade with atropine, a well-known anticholinergic drug, studying in all cases the biochemical effects.

## METHODS

### *Animals*

Experiments were performed with Sprague-Dawley rats weighing 160–240 g, having food and tap water ad lib, and maintained on 12 L:12 D cycle.

### *Intracerebral Injection*

Under pentobarbital anaesthesia (60 mg/kg), rats were injected stereotaxically twice in each striatum according to the following coordinates (in mm): Right striatum: first—A:1, L:

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2.6, V:4.5; second—A:0, L:2.6, V:4.5. Left striatum: first—A:1, L: – 2.6, V:4.5; second—A:0, L: – 2.6, V:4.5 as previously described (14). Treated rats received a total volume of 2 $\mu$l of a solution of 1.5 mg/ml FAS (FAS group) with the general procedure already described (3); controls were injected with the same volume of saline (SALINE group). Doses and coordinates were chosen considering preliminary experiments (3).

*Testing for Catalepsy*

The day of the test, rats were placed in a separate room one half to 1 h before behavioural observation to allow adaptation to the new environment. All observations were made between 1300 and 1700 h. Each rat was placed with both forepaws on a 9-cm height bar. The time taken by the rat to remove both paws from the bar (descent latency) was measured with a cutoff time of 180 s.

Rats of both groups (FAS and SALINE) were tested for catalepsy 24 h and 7 days after intracerebral injection either without any other treatment or 120 min after IP injection of HAL (0.25 mg/kg) or saline (0.1 cc).

Another group, without intracerebral injection and treated only with IP HAL, was considered as CONTROL group for behavioural studies.

To study the effects of atropine, the drug (10 mg/kg, IP) was administered to rats of FAS and SALINE groups 120 min after IP injection of HAL or saline, and catalepsy was tested 30 min before and after atropine injection. Animals were returned to their home cages between tests.

Approximately $3\frac{1}{2}$ h after IP injection, rats were decapitated and brains processed for HPLC assessment of catecholamines and metabolites.

*Endogenous Monoamine Levels*

The endogenous levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were determined by reverse-phase HPLC with electrochemical detection according to the method of Hallman and Jonsson (10). Values are expressed as ng/g of wet tissue weight.

*Drugs*

FAS-1 and 2 were isolated by gel filtration of the crude venom of *Dendroaspis angusticeps* on Sephadex G-50 and ion-exchange chromatography on Bio-Rex 70 (15). FAS-2, the one used in this study, was run on Sephadex SP-C-25, lyophilised, and dissolved in saline (12).

*Dendroaspis angusticeps* venom, DA, DOPAC, HVA, and atropine were obtained from Sigma Chemical Co.; HAL was a gift from Astra Lab. All drugs were dissolved in saline except HAL, which was dissolved in 1% lactic acid.

*Statistical Analyses*

Student's *t*-test and Wilcoxon's test were used to evaluate results.

## RESULTS

*Behavioural Studies*

*24 h.* The intrastriatal injection of FAS induced a cataleptic state (Fig. 1) that was increased by IP injection of saline or HAL (Table 1). Either with or without IP treatment, catalepsy scores showed by the FAS group were higher than those of the SALINE one. Catalepsy scores obtained in the FAS group after IP HAL were also higher than those of the CONTROL group (treated only with IP HAL) (Table 1).

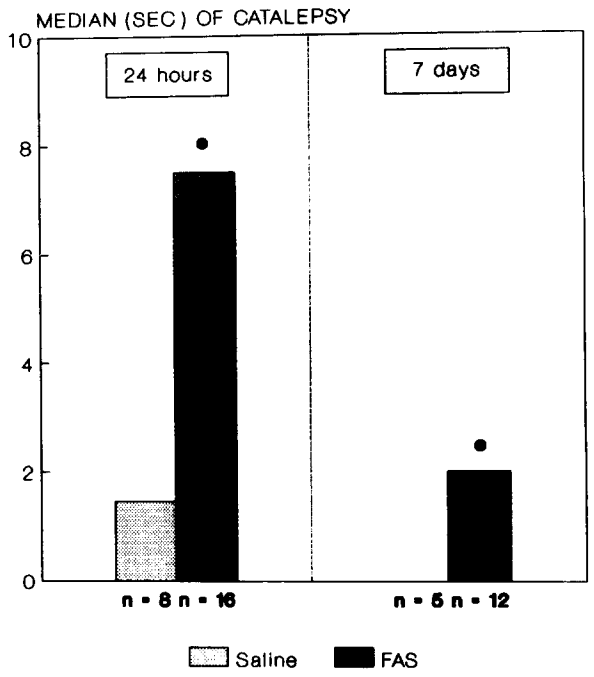


FIG. 1. Catalepsy observed in FAS and SALINE groups 24 h and 7 days after intrastriatal injection without any other treatment. Values are the median catalepsy (in seconds). *Significant change from SALINE group ($p < 0.05$).

On the other hand, rats injected intrastriatally with saline showed catalepsy only after IP HAL, which was not different from that present in the CONTROL group (Table 1).

Thirty minutes after atropine administration, the catalepsy present in FAS group after IP saline injection was reduced up to 22% of the original values, and the catalepsy present in SALINE and FAS groups after IP HAL was reduced up to 29 and 11%, respectively (Fig. 2).

*7 days.* Seven days after intrastriatal FAS injection, the catalepsy obtained without IP treatment was higher in the FAS group, although it was slightly lower than that shown in the FAS group at 24 h (Fig. 1). The cataleptic state observed 7 days after IP saline or HAL were 3 and 20 times lower than those seen at 24 h (Table 1). The catalepsy of FAS-

TABLE 1

CATALEPSY OBSERVED IN FAS AND SALINE GROUPS 24 h AND 7 DAYS AFTER INTRASTRIATAL INJECTION FOLLOWING IP TREATMENT WITH HAL (0.25 mg/kg) OR SALINE ($n$ = 7)

| Intrastriatal Treatment | None | Saline | | FAS | |
|---|---|---|---|---|---|
| Intraperitoneal Treatment | HAL | Saline | HAL | Saline | HAL |
| Descent latency | | | | | |
| 24 h | 25.5 | 0 | 39 | 69* | 98*† |
| 7 days | | 0 | 12 | 4 | 5† |

Values are the median catalepsy (in seconds) of the measures taken 120 min after IP treatment. *Significant change from rats of SALINE group under the same treatment. †Significant difference from control group (treated only with IP HAL) ($p < 0.05$).

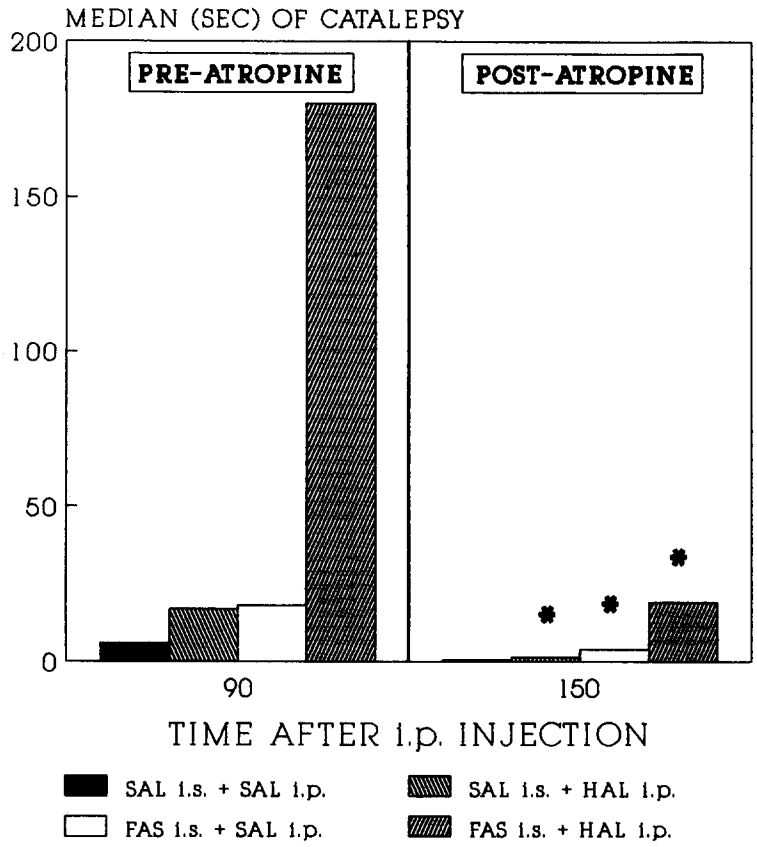


FIG. 2. Blocking effects of IP atropine upon catalepsy present after IP saline and HAL (0.25 mg/kg) in FAS and SALINE groups 24 h after intracerebral injection. Atropine (10 mg/kg) was administered 120 min after HAL. Values are the median catalepsy (in seconds) of measures registered 30 min before and after atropine administration. *Significant change from the catalepsy observed before atropine ($p < 0.05$).

HAL-treated rats was even lower than that of the CONTROL group treated only with IP HAL (Table 1). The results of the intrastriatal SALINE group were not statistically different from those seen at 24 h (Table 1).

*Biochemical Analyses*

Twenty-four hours and 7 days after intrastriatal FAS or SALINE injection, only the levels of HVA were significantly higher in the FAS group than those of the SALINE one (Table 2).

## DISCUSSION

Results showed that bilateral striatal injection of the anticholinesterase peptide FAS produced spontaneous catalepsy 24 h after surgery.

In spite of the fact that we were unable to assess acetylcholine (ACh) levels, it could be assumed that the powerful inhibition of striatal AChE provoked by FAS would produce an increase in ACh levels in the striatum similar to that found with AChE inhibitors in other experimental models (16). This increase of ACh availability in the striatum would play an important role in the catalepsy induced by FAS without HAL challenge. The potentiation by HAL of FAS-induced catalepsy would be in agreement with this hypothesis since it is known that when dopaminergic transmission is blocked by neuroleptic agents an increase in ACh liberation occurs (2).

The tendency of the treatment with IP saline to increase FAS-induced catalepsy is intriguing. It could be showing the influence of stress on the cholinergic hyperactivity evoked by FAS. The disappearance of this catalepsy in saline-treated rats 7 days after FAS could be explained by the down regulation of muscarinic receptors as discussed below.

The blockade of catalepsy with atropine, a well-known muscarinic antagonist, would be further demonstrating the role of ACh hyperactivity in the cataleptic syndrome. Therefore, our data would be in agreement with those showing that the systemic injection of cholinergic agonists such as arecoline and pilocarpine or AChE inhibitors like physostigmine produces catalepsy (13,17).

Our biochemical results showed that FAS treatment produced a significant increase only in the striatal levels of HVA. It could be hypothesized that the cholinergic hyperactivity produced by FAS increased the metabolism of dopaminergic neurones. The discussion about the site of this effect (e.g., cholinergic presynaptic receptors) would be, at the present stage of our research, only speculative.

Behavioral and biochemical results allow us then to postulate that bilateral intrastriatal injection of FAS produced a disbalance between cholinergic and dopaminergic pathways with a predominance of the former.

Bolioli et al. (3) found that the strong and long-lasting inhibition of AChE provoked by FAS was associated with a

TABLE 2

ENDOGENOUS LEVELS OF DOPAMINE AND ITS METABOLITES
24 h AND 7 DAYS AFTER INTRASTRIATAL INJECTION OF FAS OR SALINE

| DA and Metabolities | 24 h | | 7 Days | |
|---|---|---|---|---|
| | Saline (ng/g) | FAS (ng/g) | Saline (ng/g) | FAS (ng/g) |
| DA | 10130.5 ± 1773.9* | 10038.9 ± 1707.8* | 11494.8 ± 1515.4 | 11245.1 ± 2105.9 |
| DOPAC | 3733.3 ± 648.3* | 4172.6 ± 470.8* | 3343.8 ± 826* | 3800.3 ± 1379.9* |
| HVA | 3563.1 ± 980.6* | 4731.5 ± 926.9* | 2772.2 ± 570.3* | 4251.1 ± 744.9* |

Striatal levels of dopamine (DA) and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in FAS- and SALINE-treated animals after IP administration of 0.25 mg/kg haloperidol. Values are expressed as ng/g wet tissue weight and are the mean ± SD. *Significant change from SALINE group ($p < 0.05$).

downregulation of muscarinic receptors demonstrated 7 days after the unilateral striatal FAS injection. A decrease of muscarinic receptors was also demonstrated following chronic diisopropylfluorophosphate (DFP) inhibition of AChE (18). In our model, the 20-fold decrease in FAS-HAL-induced catalepsy 7 days after the intrastriatal injection would be the behavioral expression of the muscarinic receptor adaptation to the cholinergic hyperactivity induced by FAS. This fact, taken together with the atropine blockade of catalepsy, shows the critical role of muscarinic receptors in the maintenance and generation of catalepsy.

According to our results, it could be postulated that in the striatum, and at least concerning functions related to motor behaviour, the role of AChE would be mainly cholinolytic instead of noncholinergic as postulated in other regions such as the substantia nigra and locus coeruleus (1,9).

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